

THAT WHICH IS CLAIMED IS:

1. A mammalian recombination system comprising:

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- (i) FLP recombinase, or a nucleotide sequence encoding same, and
- (ii) a first DNA comprising a nucleotide sequence containing at least one FLP recombination target site therein.

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2. A recombination system according to Claim 1 further comprising:

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- (iii) a second DNA, wherein said second DNA is selected from:
 - (a) at least a second portion of said first gene of interest, or
 - (b) at least a portion of a second gene of interest;

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wherein said second DNA contains at least one FLP recombination target site; and wherein said second DNA, when combined in reading frame with said first DNA, provides a functional gene.

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3. A recombination system according to Claim 2 wherein said second DNA comprises an additional portion of said first gene of interest.

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4. A recombination system according to Claim 2 wherein said second DNA comprises at least a portion of a second gene of interest.

5. A recombination system according to Claim 4 wherein said portion of said second gene of interest,

when combined in reading frame with said first DNA,
provides a hybrid, functional gene.

6. A recombination system according to Claim 4
5 wherein said portion of said second gene of interest,
when combined with said first DNA, disrupts the function
of said first gene of interest.

7. A recombination system according to Claim 1
10 wherein said first DNA further comprises a second, FLP^{re}
recombination target site.

8. A recombination system according to Claim 1
15 wherein the FLP recombinase is derived from a species of
the genus *Saccharomyces*.

9. A recombination system according to Claim 1
wherein the FLP recombinase is derived from a strain of
Saccharomyces cerevisiae.

10. A recombination system according to Claim 9
wherein said FLP recombinase is encoded by the
approximately 1450 base pair sequence set forth as
Sequence ID No. 1.

11. A recombination system according to Claim 1
wherein said first DNA provides a readily analyzable
marker feature to the host system.

12. A recombination system according to Claim 2
30 wherein said second DNA provides a readily analyzable
marker feature to the host system.

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13. A DNA construct comprising, as an autonomous fragment:

- (a) at least one FLP recombination target site,
- 5 (b) at least one restriction endonuclease recognition site,
- (c) at least one marker gene,
- (d) a bacterial origin of replication, and optionally
- 10 (e) a mammalian cellular or viral origin of DNA replication.

14. A DNA construct comprising, as an autonomous fragment, in the following order, reading from 5' to 3' along said fragment:

- (a) a first FLP recombination target site,
- (b) an insert portion comprising, in any suitable sequence:
 - 20 (1) at least one restriction endonuclease recognition site,
 - (2) at least one marker gene,
 - (3) a bacterial origin of replication, and optionally
 - (4) a mammalian cellular or viral origin of DNA replication, and
- 25 (c) a second FLP recombination target site in tandem with said first FLP recombination target site.

15. A method for the assembly of functional gene(s), which is (are) then suitable for activation of expression in mammalian cells, by recombination of individually inactive gene segments derived from one or

more gene(s) of interest, wherein each of said segments contains at least one recombination target site, said method comprising:

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contacting said individually inactive gene segments with a FLP recombinase, under conditions suitable for recombination to occur, thereby providing a DNA sequence which encodes a functional gene of interest.

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16. A method according to Claim 15 wherein the FLP recombinase is derived from a species of the genus *Saccharomyces*.

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17. A method according to Claim 15 wherein the FLP recombinase is derived from a strain of *Saccharomyces cerevisiae*.

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18. A method according to Claim 17 wherein said FLP recombinase is encoded by the approximately 1450 base pair sequence set forth as Sequence ID No. 1.

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19. A method for the disruption of functional gene(s) of interest, rendering said gene(s) unable to be inactivated for expression in mammalian cells wherein said gene(s) of interest contain at least one FLP recombination target site, said method comprising contacting said gene(s) of interest with:

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(i) a DNA segment which contains at least one FLP recombination target site, and

(ii) FLP recombinase;

wherein said contacting is carried out under conditions suitable for recombination to occur between said gene and

said DNA segment, thereby disrupting the gene(s) of interest and rendering said gene(s) non-functional.

20. A method according to Claim 19 wherein said
5 DNA segment provides a readily analyzable marker feature to the host system.

21. A method according to Claim 19 wherein the
10 FLP recombinase is derived from a species of the genus *Saccharomyces*.

22. A method according to Claim 19 wherein the
15 FLP recombinase is derived from a strain of *Saccharomyces cerevisiae*.

23. A method according to Claim 22 wherein said
FLP recombinase is encoded by the approximately 1450 base pair sequence set forth as Sequence ID No. 1.

20 24. A method for the recovery of transfected DNA from the genome of a transfected organism, wherein the genomic DNA of said transfected organism contains a fragment having two tandemly oriented FLP recombination target sites therein, said method comprising contacting
25 genomic DNA from said organism with FLP.

25. A method for the precisely targeted integration of DNA into the genome of a host organism, said method comprising:

30 (i) introducing a FLP recombination target site into the genome of cells which are compatible with the cells of the subject,

- (ii) introducing a first DNA comprising a nucleotide sequence containing at least one FLP recombination target site therein into the FLP recombination target site in the genome of said cells by contacting said cells with said first DNA and FLP recombinase, and thereafter
- (iii) introducing the cells produced by the process of step (ii) into said subject.

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26. A method according to Claim 25, further comprising contacting the genomic DNA from said subject with FLP, thereby recovering the transfected DNA containing said first gene of interest from the genome of said transfected organism.

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27. A method according to Claim 26, further comprising introducing at least a portion of a second gene of interest into said FLP recombination target site.

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28. A method according to Claim 25, further comprising introducing at least a portion of a second gene of interest into one of the FLP recombination target sites of said subject.

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29. A mammalian cell, wherein the genomic DNA of said cell contains at least one FLP recombination target site therein.

30. A mammalian cell according to Claim 29 wherein said FLP recombination target site in the genomic DNA of said cell is positioned within at least a portion of one or more gene(s) of interest.

31. A mammalian cell according to Claim 30, further comprising DNA encoding, and capable of expressing, in mammalian cells, a FLP recombinase.

5 32. A mammalian cell according to Claim 30 wherein said gene(s) of interest provide a readily analyzable marker feature to the host system.

10 33. A mammalian cell according to Claim 29 wherein said FLP recombination target site has the sequence:

5'-GAAGTTCCTATTCTCTAGAAAGTATAGGAAGTT₁

C-3'SEQ ID NO. 3

15 or functional equivalents thereof.

34. A mammalian cell according to Claim 30 further comprising an additional DNA fragment, wherein said additional DNA fragment is selected from:

- 20 (a) at least a second portion of said first gene of interest, or
(b) at least a portion of a second gene of interest;

wherein said second DNA contains at least one FLP recombination target site; and wherein said second DNA, when combined in reading frame with said first DNA, provides a functional gene.

35. A transgenic, non-human mammal, wherein said mammal contains at least one FLP recombination target site in the genomic DNA thereof.

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36. A transgenic, non-human mammal according to Claim 35 wherein said FLP recombination target site is positioned within at least a portion of one or more gene(s) of interest.

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37. A transgenic, non-human mammal according to Claim 35, further comprising a nucleotide sequence encoding, and capable of expressing, in transgenic, non-human mammals, a FLP recombinase.

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38. A transgenic, non-human mammal according to Claim 35, further comprising FLP recombinase.

39. A transgenic, non-human mammal according to Claim 36 wherein said gene(s) of interest provide a readily analyzable marker feature to the host system.

40. A transgenic, non-human mammal according to Claim 35 wherein said FLP recombination target site has the sequence:

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C-3 SEQ ID NO:3)

5'-GAAGTTCTATTCTCTAGAAAGTATAGGAACTT

or functional equivalents thereof.

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41. A transgenic, non-human mammal according to Claim 36 further comprising an additional DNA fragment, wherein said additional DNA fragment is selected from:

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- (a) at least a second portion of said first gene of interest, or
- (b) at least a portion of a second gene of interest;

wherein said second DNA contains at least one FLP recombination target site; and wherein said second DNA, when combined in reading frame with said first DNA, provides a functional gene.

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42. A method for the site-specific integration of transfected DNA into the genome of a cell according to Claim 29, said method comprising:

- 5 (i) contacting said genome with:
- (a) FLP recombinase, and
- (b) a first DNA comprising at least a portion of a first gene of interest;
- 10 wherein said first DNA contains at least one FLP recombination target site; and thereafter
- (ii) maintaining the product of step (i) under conditions suitable for site-specific integration of said DNA sequence to occur at the FLP recombination target site in
- 15 said genome of the host cells.

43. A method according to Claim 42 wherein said FLP recombination target site in the genomic DNA of said cell is positioned within at least a portion of one or

20 more gene(s) of interest.

44. A method according to Claim 42 further comprising additionally contacting said host cell with a second DNA, wherein said second DNA is selected from:

25 (a) at least a second portion of said first gene of interest, or

(b) at least a portion of a second gene of interest;

wherein said second DNA contains at least one FLP recombination target site; and wherein said second DNA, when combined in reading frame with said first DNA, provides a functional gene.

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45. A method according to Claim 42 wherein said

35 FLP recombinase is provided by a FLP expression vector.

46. A method according to Claim 45 wherein the expression of FLP recombinase by said FLP expression vector is subject to regulatory control.

5 47. A method according to Claim 42 wherein said FLP recombination target site is introduced into the genome of said host mammalian cell by transfecting said host cell with a DNA fragment containing at least one recombination target site therein.

10 48. A method according to Claim 42 wherein the FLP recombination target site in the genomic DNA of said host mammalian cell is so positioned that the introduction of additional DNA sequences therein will
15 inactivate the target gene.

49. A method for the site-specific integration of transfected DNA into the genome of a host according to Claim 35, said method comprising:

- 20 (i) contacting said genome with:
(a) FLP recombinase, and
(b) a first DNA comprising a nucleotide sequence containing at least one FLP recombination target site therein;
25 and thereafter
(ii) maintaining the product of step (i) under conditions suitable for site-specific integration of said DNA sequence to occur
30 at the FLP recombination target site in said genome of the host.

50. A method according to Claim 49 wherein said FLP recombination target site is positioned within at least a portion of one or more gene(s) of interest.

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51. A method according to Claim 49 further comprising additionally contacting said host with a second DNA, wherein said second DNA is selected from:

- 5 (a) at least a second portion of said first gene of interest, or
(b) at least a portion of a second gene of interest;

wherein said second DNA contains at least one FLP recombination target site; and wherein said second DNA, when combined in reading frame with said first DNA, provides a functional gene.

52. A method according to Claim 49 wherein said FLP recombinase is provided by a FLP expression vector.

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53. A method according to Claim 52 wherein the expression of FLP recombinase by said FLP expression vector is subject to regulatory control.

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54. A method according to Claim 49 wherein said FLP recombination target site is introduced into the genome of said host mammal by transfecting said host with a DNA fragment containing at least one recombination target site therein.

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55. A method according to Claim 49 wherein the DNA of said host mammal contains at least one FLP recombination target site, and wherein said FLP recombination target site is so positioned that the introduction of additional DNA sequences therein will inactivate the target gene.

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56. A method for the analysis of the development of a mammal, said method comprising:

(a) providing a transgenic mammal comprising:

- 5 (i) an expression construct encoding FLP under the control of a conditional promoter, and
- 10 (ii) a reporter construct under the control of the same or a different promoter, wherein said reporter construct encodes a functional or non-functional reporter gene product, and wherein said construct contains at least one FLP recombination target site therein,
- 15 wherein the functional expression of the functional reporter gene is disrupted when said FLP recombination event occurs, or
- 20 wherein the functional expression of the non-functional reporter gene commences when said FLP recombination event occurs; and

(b) following the development of said mammal to determine when expression of functional reporter gene product either commences or is disrupted.

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57. A method according to Claim 56 wherein said conditional promoter is developmentally-regulated.

30 58. A co-transfection assay for the occurrence of FLP-mediated recombination, said assay comprising:

(a) co-transfecting a host mammalian cell with:

- 35 (i) a FLP expression plasmid, and
- (ii) a reporter plasmid comprising a non-functional reporter gene wherein said non-functional reporter gene is inactivated by

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the presence of extraneous DNA containing
at least one recombination target site;
and

(b) monitoring said host cell under a variety
5 of conditions for the gain of expression of functional
reporter gene product.

59. A co-transfection assay for the occurrence
of FLP-mediated recombination, said assay comprising:

10 (a) co-transfecting a host mammalian cell
with:

(i) a FLP expression plasmid, and
(ii) a reporter plasmid comprising a
functional reporter gene containing at
15 least one recombination target site
therein; and

(b) monitoring said host cell under a variety
of conditions for the loss of expression of functional
reporter gene product.

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